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13. ABSTRACT *(Maximum 200 words)*

This cooperative Augmentation Award for Science and Engineering Research Training has provided training for two doctoral students in several important areas of marine microalgal biofouling, including marine diatom mass culturing and adhesive isolation, biochemistry of adhesives, and laboratory experiments on the effects of substrate preconditioning on diatom adhesion. Students have gained significant experience through exchange between cooperating labs. This grant augmented research described in "parent" award #N00014-94-1-0273. The overall goal of this research has been to provide a better understanding of the diatom portion of the biofouling process, to facilitate the development of more effective methods to control marine biofouling. We have identified bromide as an absolute requirement for the formation of the principal attachment organ for a common biofouling diatom, which provides a unique and powerful tool for initiating synchronous stalk production on a large scale and facilitating further extracellular adhesive biosynthesis, secretion and physiology research. Several monoclonal antibodies directed against the adhesive of *Achnanthes* were produced, allowing for effective isolation, localization and analysis of adhesive components. Detailed chemical characterization of antibody epitopes in adhesives from *Achnanthes* provides requisite information to determine the nature of the interactions responsible for assembly and function of the adhesives.

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FINAL REPORT

Grant#: N00014-94-1-00766

PRINCIPAL INVESTIGATORS: Michael R. Gretz and Kyle D. Hoagland

INSTITUTION: Michigan Technological University; University of Nebraska

ASSERT GRANT TITLE: Molecular and Physiological Mechanisms of Diatom Fouling Phenomena

AWARD PERIOD: 1 July 1994 - 30 September 1997

OBJECTIVES: This Augmentation Award for Science and Engineering Research Training supported two additional graduate students on research described in "parent" award # N00014-94-1-0273. The objectives of the AASERT project have been to: (1) complete a biochemical analysis of the adhesives of two major marine fouling diatoms, including genera representing each of the two principal attachment types, a capsule former (*Amphora coffeaeformis*) and two stalk formers (*Achnanthes longipes* and *Cymbella cistula*); and (2) investigate the physiology of biosynthesis and secretion of adhesives and external factors that control adhesive formation.

The overall goal of this research has been to provide a better understanding of the diatom portion of the biofouling process, to facilitate the development of more effective methods to control marine biofouling. This cooperative project was designed to provide training for two doctoral students in several important areas of marine microalgal biofouling, including marine diatom mass culturing and adhesive isolation, biochemistry of adhesives, and laboratory experiments on the effects of substrate preconditioning on diatom adhesion.

APPROACH: *Achnanthes longipes, Cymbella cistula*, and *Amphora coffaeaformis* were mass cultured to produce large quantities of stalk and capsular EPS in defined medium. The effects of growth conditions on stalk production were investigated using gradients of numerous parameters, including nutrients, nutrient ratios, heavy metals, temperature, pH, light quality, and inoculum cell density. *Lotus*-FITC, a fucose-binding lectin was used in conjunction with a fluorescence microscope-image analysis system, to quantify *Achnanthes* stalks and EPS secretion along these gradients. We have developed and utilized specific molecular probes directed against the adhesives, including mono- and poly-clonal antibodies, lectins and enzymes. Biochemical analysis of small quantities of extracellular material was conducted to identify the adhesive and provide information as to the epitopes conferring specificity of the above probes.

ACCOMPLISHMENTS: The project has been somewhat unique in that opportunities for training in this broad range of topics were accomplished in part by an exchange of doctoral students among cooperating labs. Brandon Wustman, a Ph.D. student from Michigan Technological University visited the University of Nebraska to participate in studies on lectin localization of stalk polysaccharide components in *A. longipes*, while Lisa Johnson from University of Nebraska (Ph.D. 1995) worked at the electron microscopy laboratory at the University of Wisconsin using cryo-preservation and cryo-microscopy techniques to localize bromide in *A. longipes* stalks and cells. Three major publications, one Ph.D. dissertation and numerous abstracts (one

receiving the Bold Award for best student paper) were produced by students supported by AASERT funds. The major findings from this research are summarized below:

The common biofouling diatom *A. longipes* was shown to exhibit an absolute requirement for bromide for stalk production and substratum attachment, with optimal stalk production at 30 mM bromide (Johnson, 1995; Johnson, *et al.* 1995). Varying EPS morphologies resulted from altering bromide and iodide levels. Cells grown in elevated iodide secreted significantly more soluble extracellular carbohydrate, indicating that the EPS was soluble and unable to polymerize into a stalk. Replacing sulfate with methionine resulted in loss of ability to form stalks even in the presence of bromide, suggesting that sulfate is required for cross-linking of stalk polymers.

Preliminary evidence indicated that stalk production in *A. longipes* is dependent upon the inoculum cell density, suggesting a quorum effect previously undescribed in diatoms. Experiments are currently underway to further define this effect.

A. longipes produces stalks that are differentially localized by cytochemical stains and sugar specific lectins (Wustman *et al.* 1997) and with the multiple mono- and poly-clonal antibodies we have raised against native stalks of *A. longipes* (Wustman *et al.* 1998), providing evidence for chemically unique regions. The inner core region contains primarily sulfated polysaccharides (Johnson, 1995) while the three outer regions contain polymers with a predominance of carboxylated residues. Components of the inner and outer stalk layers are fucoglucuronogalactans associated with protein moieties via O-glycosidic linkages (Wustman *et al.* 1998). We have extracted these molecules and subfractionated them into three size ranges: 1) >20,000,000 MW; 2) ≅ 100,000 MW, and; 3) < 10,000 MW. We have successfully generated antibodies with high affinities for components within each of these fractions (Wustman *et al.* 1998).

In contrast to *A. longipes*, *C. cistula* produces stalks that are uniformly localized by cytochemical stains and sugar specific lectins (Wustman *et al.* 1997). The stalk of *C. cistula* does not contain discernable substructure and can be completely solubilized with EDTA or CDTA suggesting a cationic cross bridging of molecules. Preliminary chemistry indicates that *C. cistula* adhesives consist of a sulfated xylogalactan (Wustman *et al.* 1997) and several proteins/glycoproteins of ~ 60 kD. Calcium and/or magnesium most likely play an important role in the initial aggregation of anionic extracellular polymers of both diatoms. EDTA does not dissolve or produce any observable physical affects on extracellular adhesives of *A. longipes*, indicating that, if cationic cross bridging of anionic polymers is an important mechanism involved in assembly of extracellular adhesives, it is not the only one.

SIGNIFICANCE: Identification of bromide as an absolute requirement for the formation of the principal attachment organ for a common biofouling diatom provides a unique and powerful tool for initiating synchronous stalk production on a large scale and facilitating further extracellular adhesive biosynthesis, secretion and physiology research. Several monoclonal antibodies directed against the adhesive of *Achnanthes* provide powerful tools for isolation, localization and analysis of adhesive components. Detailed chemical characterization of antibody epitopes provides requisite information to determine the nature of the interactions responsible for assembly and action of the adhesives.

AWARD INFORMATION: Brandon Wustman, Ph.D. candidate at MTU, received the Bold Award for best student presentation at the International Phycological Congress, Leiden, The Netherlands, 1997.

PUBLICATIONS, THESES AND ABSTRACTS (1994-97):

Wustman, B.A., Wetherbee, R., Lind, J. and Gretz, M.R. 1998. Extracellular matrix assembly in diatoms (Bacillariophyceae) III. Organization of fucoglucuronogalactans within the adhesive stalks of Achnanthes longipes. Plant Physiol. [in press].

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Wustman, B.A. and Gretz, M.R. 1997. Modeling extracellular adhesives of *Achnanthes longipes* and *Cymbella cistula* (Bacillariophyceae). Phycologia 36(suppl.):127(abst.) Int. Phycol. Congress, Leiden, The Netherlands (Bold Award)

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Johnson, L.M., K.D. Hoagland and M.R. Gretz (1995) Effects of bromide on stalk secretion in *Achnanthes longipes*. J. Phycol. (suppl.)31:22 (abst.)

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Wustman, B.A., Gretz, M.R. and Hotchkiss, A.T. 1994. Uronic acid containing diatom mucilages: evaluation of three methods for characterization of acidic polysaccharides. (abst.) ASLO/PSA, Miami